Mycologia, 102(2), 2010, pp. 374–383. DOI: 10.3852/09-093

Ulocladium cantlous sp. nov. isolated from northwestern China: its morphology and molecular phylogenetic position

Yong Wang
Yun-Fei Pei
Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, China

Nichole R. O'Neill
United States Department of Agricultural, Agriculture Research Service, Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland 20705-2350

Xiu-Guo Zhang[1]
Department of Plant Pathology, Shandong Agricultural University, Taian, 271018, China

Abstract: A new species of *Ulocladium* was isolated from diseased leaves from two *Cucumis* sp. growing in Sinkiang and Gansu provinces of China. Conidia were isolated from necrotic leaves and used to establish single-spore pure cultures. Conidia were harvested from cultures 7 d after incubation for morphological comparisons. The morphology of this species resembles that of *U. botrytis* and *U. consortiale*. However it is distinguished from these two species by the sizes of obovoid to broadly ellipsoidal conidia and longer conidiophores. A taxonomic description of *U. cantlous*, comparison with related species in this genus, and a species phylogeny based on the partial nucleotide sequence of the glyceraldehyde-3-phosphate dehydrogenase (*gpd*) gene and the *Alternaria alternata* major allergen (Alt a 1) gene are provided.

Key words: hyphomycetes, new species, systematics

INTRODUCTION

Genus *Ulocladium* was created by Preuss in 1851 with *U. botrytis* designated as type species. This name subsequently was largely ignored because its component taxa shared several characteristics with *Alternaria* and *Stemphylium*, even though some species in these genera could be separated from other genera by the juvenile and mature shapes of conidia, as well as by the modes of proliferation of conidiophores and conidia. Based on the re-examination of diagnostic morphology for *Ulocladium*, *Alternaria* and *Stemphylium*, Simmons (1967) re-established genus *Ulocladium* to accommodate phaeodictyosporic taxa with primarily obovoid, nonbeaked conidial shapes. Moreover he observed a group of species at different growth stages under standardized conditions and found that four species in the *U. atrum* group (*U. atrum*, *U. cucurbitae*, *U. multiforme* and *U. dauci*) possessed similar multiplex conidium morphologies (i.e. conidia produced during the early 1–3 d growth often had a strikingly different morphology than those produced at 4–7 d) (Simmons 1982, 1998). More than 20 species of *Ulocladium* have been reported to date (http://www.indexfungorum.org/Names/Names.asp).

Some species of *Ulocladium* have a widespread distribution and occur in soil as saprophytes, playing an important ecological role in the decomposition and recycling of materials in natural ecosystems. Some species are colonizers in buildings after floods or other water damage, and some species are cellulolytic, often growing on damp wood and paper (Grishkan et al. 2007, Saparrat et al. 2007). A few species are plant pathogens that cause a range of diseases on important agricultural crops and fruit trees (Vannini and Vettraino 2000, Zitter and Hsu 1990). One species suppresses sporulation of *Botrytis* sp. by competitive saprophytic colonization of leaf tips of onion or necrotic leaf tissues of lily canopies (Elmer and Köhl 1998, Köhl et al. 2003).

The taxonomy of genus *Ulocladium* is based mainly on morphology, including conidial shape, sizes, septation, ornamentation and presence or absence of catenulation by means of apical secondary conidiophores (Simmons 1967, 1990). These morphological characters are still indispensable in identifying a new taxon. However some morphological characteristics of *Ulocladium* spp. vary depending on culture conditions. Conidial characteristics of *U. cucurbitae* and *U. chartarum* vary on different substrates and at different temperatures (Leach and Aragaki 1970, Simmons 1982). Simmons and Roberts (1993) proposed that the best morphological criterion for small-spored *Alternaria* taxa (similar to *Ulocladium*) was to compare the elements of sporulating isolates under standardized conditions. Morphological features, for example size range and septation, overlap among *Ulocladium* species, causing difficulty in establishing new taxa (de Hoog and Horré 2002).

The morphological descriptions of species are increasingly being supplemented by gene sequences.

Submitted 29 Apr 2009; accepted for publication 25 Jul 2009.
[1] Corresponding author. E-mail: zhxg@sdau.edu.cn

Phylogenetic analysis has become a common tool for the separation, identification and determination of species and their natural relationships and for ascertaining genetic diversity. Recent molecular analyses have largely improved our understanding of the phylogenetic relationships among *Ulocladium* species (Hong et al. 2005, Pryor and Bigelow 2003, Runa et al. 2009, Xue and Zhang 2007). Glyceraldhyde-3-phosphate dehydrogenase (*gpd*) gene sequences were used extensively in the resolution of phylogenetic relationships among *Alternaria/ Lewia, Bipolaris/ Cochliobulus, Drechslera/Pyrenophora* and *Stemphylium/ Pleospora* (Berbee et al. 1999, Câmara et al. 2002, Pryor and Bigelow 2003, Zhang and Berbee 2001). The relatively high level of variability in the *gpd* gene sequence (Smith 1989) facilitates phylogenetic comparisons among species and provides better discrimination of species than rDNA ITS regions (Berbee et al. 1999).

The major allergen produced by *Alternaria alternata*, Alt a 1, is a protein with no known function in fungal metabolism or ecology (Barnes et al. 1996, de Vouge et al. 1996). A homolog of Alt a 1 later was found to be highly up-regulated during the infection of *A. brassicicola* on *Arabidopsis thaliana*, suggesting that this protein might be involved in plant pathogenicity (Cramer and Lawrence 2003, 2004). Alt a 1 also is expressed in other members in the Pleosporaceae, including *Stemphylium* sp., *Ulocladium* sp. and *Curvularia* sp. (Sáenz-de-Santamaría et al. 2005). Thus the gene encoding this protein might be useful in reconstructing phylogenetic relationships among *Ulocladium* species. As a molecular marker to study phylogenetic relationships, Alt a 1 exon regions contained considerably more parsimony informative sites than ribosomal genes (rDNA ITS, mitochondrial small subunit [mtSSU] rDNA) and other protein-coding genes (*gpd*) (Cramer and Lawrence 2003). Although some differences in relationships among certain species and long-branched terminal taxa were discovered based on the phylogenetic analyses of Alt a 1 and *gpd* genes, many phylogenetic features of these two genes are similar (Hong et al. 2005). The combination of these two datasets provides more sequence data for identifying a taxon than would be possible with either alone.

An undescribed *Ulocladium* sp. was isolated from the diseased leaves of *Cucumis melo* L. and *C. sativus* L. in northwestern China. The morphology of this species was typical for genus *Ulocladium*, and it was similar to *U. botrytis* and *U. consortiale* in producing obovoid or ellipsoid conidia. However distinctive taxonomic characters, together with the phylogeny based on partial nucleotide sequences of the Alt a 1 and *gpd* genes, prompted the description of a new species.

MATERIALS AND METHODS

Isolations of fungi.—Diseased leaves of *Cucumis melo* L. and *C. sativus* L. were collected from Sinkiang and Gansu provinces in northwestern China. Spores were taken from areas with necrotic leaf spots of field-collected leaves, and single spores were isolated on potato-dextrose agar (PDA; 20 g white potato boiled and filtered, 20 g dextrose, 20 g agar, 1 L distilled water). The isolates were transferred from PDA to potato-carrot agar (PCA; 20 g white potato boiled and filtered, 20 g carrot boiled and filtered, 20 g agar, 1 L distilled water). Mycelial plugs cut from leading edges of colonies on PDA were stored on PDA slants at 4 C. The holotype (dried culture) of this new species was deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP). Living cultures of each isolate were deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP), and the collection of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.

Morphological and cultural studies.—Two isolates ($HSAUP_{wy}0209$, $HSAUP_{wy}0526$) of the *Ulocladium* sp. were grown on PDA. Morphological comparisons among these isolates and eight similar species (*U. atrum, U. botrytis, U. consortiale, U. cucurbitae, U. dauci, U. multiforme, U. obovoideum* and *U. subcucurbitae*; TABLE II) were based on living cultures grown under standardized conditions on potato-carrot agar (PCA) in plastic Petri dishes under ambient temperature (20–23 C) and cool-white fluorescent light 35–40 cm above the culture, with an 8 h on and 16 h off cycle (Simmons 1998, Simmons and Roberts 1993). Samples of conidia for comparison and photography were taken from 7 d old colony. Fifty mature conidia and 30 conidiophores were measured with a Nikon 90i microscope (Nikon Corp., Japan) at 100× magnification. The morphology of conidia and conidiophores in lactic acid was recorded by light microscopy and photographed.

DNA extraction, PCR amplification and sequencing.—DNA extraction and purification were conducted according to the protocols of Pryor and Gilbertson (2000). Amplification of portions of Alt a 1 and *gpd* genes were conducted with the primers that were designed based on the conserved regions of Alt a 1 and *gpd* genes in *U. atrum, U. botrytis, U. chartarum* and *U. cucurbitae* (Hong et al. 2005). Reaction mixtures contained 5 μL 10× ThermoPol reaction buffer (200 mM Tris-HCl, pH 8.3, 100 mM KCl, 100 mM $[NH_4]_2SO_4$, 20 mM $MgSO_4$ and 1% Triton X-100), 5 μL 10 mM $MgSO_4$, 20 ng template genomic DNA, 4 pM each primer, 4 μL 2.5 mM dNTP, 0.5 U AmpliTaq polymerase, and total volume was adjusted to 50 μL with deionized water. PCR amplification of Alt a 1 and *gpd* regions was conducted with reaction conditions described by Hong et al. (2005). The PCR-amplified DNA fragments were fractionated in 1.0% agarose gels in 0.5× TBE buffer, and DNA was viewed by ethidium bromide staining and UV illumination. PCR products were purified with DNA fragment Purification Kit 2.0 (TakaRa). They were cloned into the pEASY-T3 vector following the manufacture's protocol of the pEASY-T3 Cloning Kit (China) and then

TABLE I. Isolates used in this study for morphological and molecular analysis

Species	Source[a]	GenBank accession numbers	
		Alt a 1	*gpd*
Alternaria cheiranthi	EGS 41-188	AY563290	AY278802
Embellisia indefessa	EGS 30-195	AY563323	AY278828
Stemphylium botryosum	ATCC 42170	AY563274	AY278820
Ulocladium alternariae	BMP 0352	AY563316	AY278815
Ulocladium sp.	CBS 123375	EU862546	EU862547
U. atrum	CBS 195.67	AY563318	AY278818
U. botrytis	CBS 198.67	AY563317	AY278817
U. cantlous	CBS 123007	**EU684146**[b]	**EU684145**
	CBS 124653	**EU684150**	**EU684153**
U. capsicuma	CBS 102062	**EU684149**	AY762950
U. chartarum	CBS 200.67	AY563319	AY278819
U. consortiale	CBS 123806	FJ008714	FJ008717
U. cucurbitae	EGS 31-021	AY563315	AY562418
U. dauci	CBS 102062	FJ266510	FJ266495
U. multiforme	CBS 102060	FJ266512	FJ266497
U. obovoideum	CBS 101229	**EU684147**	**EU684151**
U. oudemansii	CBS 137. 81	**EU684148**	**EU684152**
U. septosporum	CBS 109.38	FJ266515	FJ266500
U. subcucurbitae	CBS 121491	**EU855807**	**EU855803**

[a] Source abbreviations: ATCC = American Type Culture Collection, Manassas, Virginia. BMP = Division of Plant Pathology and Microbiology, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721. CBS = Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. EGS = E. G. Simmons, Mycological Services, Crawfordsville, IN 47933.

[b] Sequences that were determined in this study appear in boldface.

transformed into DH5α chemically competent *Escherichia coli*. Sequencing was performed with an ABI PRISM 3730 DNA auto-sequencer with either dRhodamine terminator or Big Dye Terminator chemistry (Applied Biosystems Inc., Foster City, California). The sequences of both strands of each fragment were determined for sequence confirmation. The DNA sequences of Alt a 1 and *gpd* regions generated in this study were submitted to GenBank (accession numbers EU684145–EU684149, EU684150–EU684153, EU855803, EU855807), and additional DNA sequences representing 14 other species were downloaded from GenBank (TABLE I).

Molecular phylogenetic analyses of Alt a 1 and gpd *genes.*—Sequences were aligned manually with SEQUENCHER 3.1 (Gene Codes, Ann Arbor, Michigan) and Clustal X 1.81 (Thompson et al. 1997). The alignments were checked visually, improved manually where necessary and deposited in TreeBASE (SN4573-23198). In the analyses alignment gaps were treated as missing data. Phylogenetic analyses were performed with PAUP 4.0b10 (Swofford 2002). Trees were produced with neighbor joining (NJ) and maximum parsimony (MP) analyses of combined *gpd* and Alt a 1 sequence datasets. The Kimura two-parameter distance calculation was used in NJ analysis. In the MP analyses trees were inferred with heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions. MAXTREES were 100, branches of zero length were collapsed and all parsimonious trees were saved. Characters were treated as unweighted in the analysis, and gaps were treated as missing data. Partition homogeneity test (Farris et al. 1995, Huelsenbeck et al. 1996) was used to determine whether these two datasets (*gpd* and Alt a 1) could be combined, and a combined analysis was run with the parameters described above.

TAXONOMY

Ulocladium cantlous Yong Wang bis & X.G. Zhang, sp. nov. FIG. 1A–F

MycoBank MB 512259

Coloniae in agaro PCA descripta. Coloniae cinereo-albae vel atro-brunneae, auctus velosi, sporulatione abunda, conspicue concentrice zonata. Hyphae vegetativae septatae, ramosae, subhyalinae vel dilute brunneae, 2.5–3 μm crassae, laeves. Conidiophora copiosa, recta vel acclivia, simplicia vel ramosa, ex lateribus hypharum praecipue submersarum oriunda, dilute aureo-brunnea, septata, 3–4 μm crassa, plerumque 80–130 μm longa, 5–10 genicula sporifera praebentia. Conidia in colonia plerumque obovoidea vel crassa ellipsoidea, ad basim subacuta vel conica, apicem crassa rotundata, subacuta vel conica, pallide-brunneae vel medio-brunneae, 24–36 × 13–15 μm, vulgo 3–4 transverse septata et 0–2 longiseptata, verrucosa vel dense verrucosa. Conidiophora secundaria saepe longiuscula, ad ca. 5–10 μm, 1–2 apicaliter geniculata et conidiogena.

Colonies on PCA yellow brown to dark brown, rapidly covering the Petri dish within 7 d. Concentric zonation of growth pronounced, and sporulation on

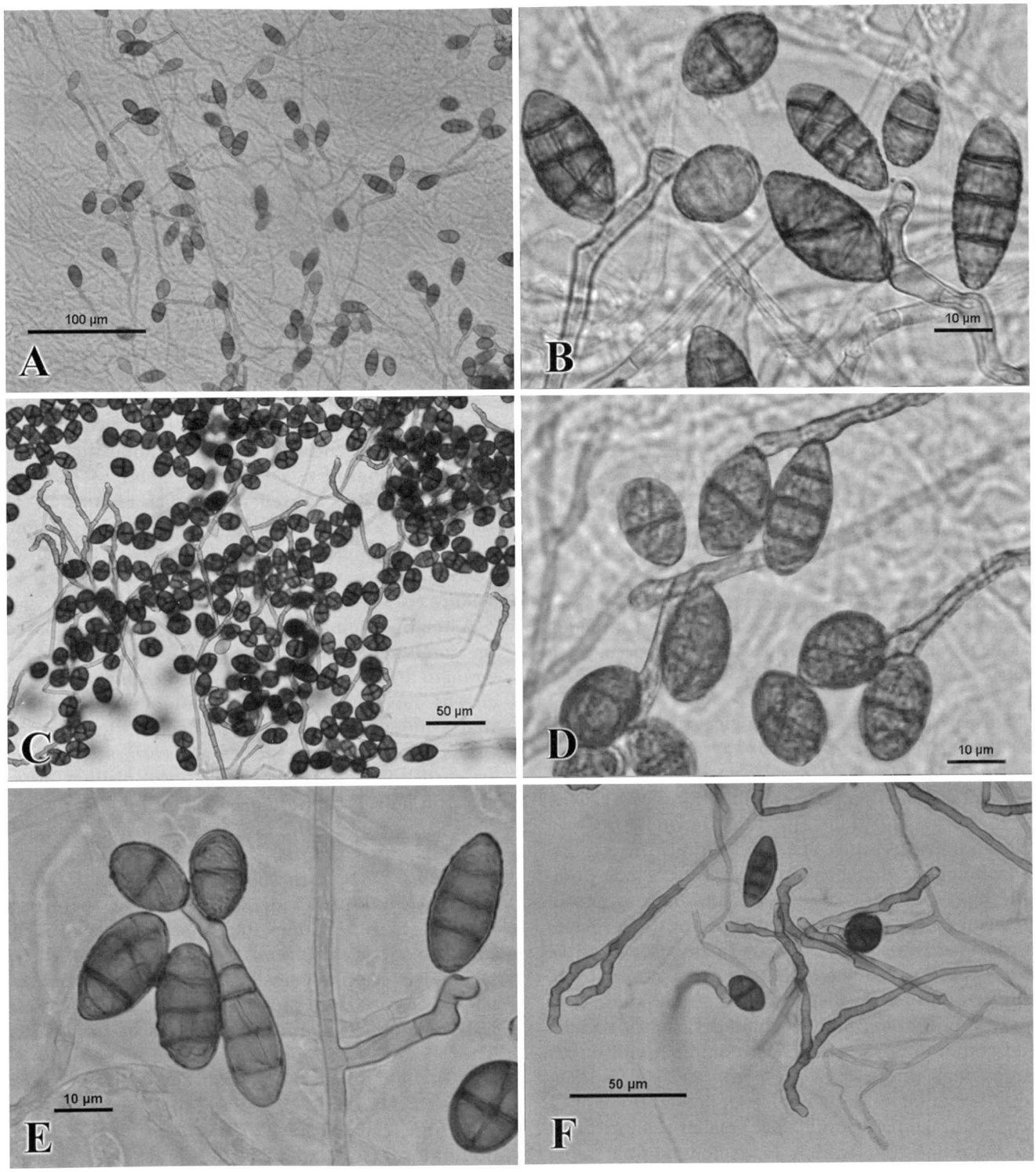

FIG. 1. *Ulocladium cantlous*. A. Primary obovoid or broadly ellipsoidal conidia at 1–3 d. B. Details of primary conidia at this stage. C. Mature obovoid or broadly ellipsoidal conidia at 4–7 d. D. Details of mature conidia at this stage. E. Conidia with secondary conidiophores. F. Characteristic pattern of conidiophores.

this medium abundant. Mycelium subhyaline to dilute yellowish brown, hyphae branched, smooth, septate and 2.5–3 µm wide. Conidiophores abundant, erect or ascending from submerged and aerial hyphae, simple or branched, dilute golden brown, septate, 80–130 × 3–4 µm (av. 105 × 3.5 ± 0.2 µm), with 5–10 perforate geniculations (FIG. 1F). Conidia mostly obovoid or broadly ellipsoidal: base subacute

to conical, apex broadly rounded or subacute to conical (FIG. 1A–D). They commonly are 24–36 × 13–15 µm (av. 29 ± 3.7 × 14 ± 0.4 µm), usually 3–4 transverse septa, 0–2 short longitudinal septa. Conidia pale brown to medium brown; walls verrucose to densely verrucose (FIG. 1B, D). Conidia solitary or occasionally generate short secondary conidiophores (FIG. 1A, E). Secondary conidiophores up to 5–10 µm long with 1–2 conidiogenous sites (FIG. 1E). These conidia with secondary spores (i.e. young ellipsoidal and ovoid spores) on their secondary conidiophores (FIG. 1E).

Holotype. Dried culture of $HSAUP_{wy}0209$, after 7 d on PCA medium, deposited in Herbarium of the Department of Plant Pathology, Shandong Agricultural University, CHINA. The isolate derived from diseased leaves of *Cucumis melo* from a farm of Kashi, Sinkiang, China, Y. Wang, 20 Jul 2006. *Ex-type* culture: $HSAUP_{wy}0209$; CBS 123007.

Etymology. Referring to the host (cantaloupe), from which this fungus was isolated.

Known distribution. Sinkiang and Gansu provinces, China.

Additional specimens examined. $HSAUP_{wy}0526$ collected from the diseased leaves of *Cucumis sativus* in Lanzhou of Gansu province, Y. Wang, 30 Jul 2006. A living culture is stored in the Centraalbureau voor Schimmelcultures, CBS 124653.

This species shares morphological similarities with *U. atrum*, *U. botrytis*, *U. consortiale*, *U. cucurbitae*, *U. dauci*, *U. multiforme*, *U. obovoideum* and *U. subcucurbitae*, all of which produce conidiophores with closely perforate geniculations and fundamentally obovoid conidia. These nine species exhibit the typical morphological characters of *Ulocladium*, which probably represent basic core characters for this genus. Detailed morphological information for these species is provided (TABLE II), but only *U. consortiale* and *U. botrytis* possess characters that are most similar to *U. cantlous*, which is distinguished from these two species morphologically by characteristics of conidia and conidiophores. *U. cantlous* conidia are distinctly larger than those of *U. botrytis*, and *U. cantlous* conidia exhibit more transverse septa than those of *U. botrytis*. *U. cantlous* conidia are smaller than those of *U. consortiale* and exhibit fewer longitudinal septa than *U. consortiale*. The ornamentation of conidial walls also distinguishes *U. cantlous* (verrucose to densely verrucose) from *U. consortiale*, which has closely micromaculate to definitely verrucose conidial walls. The conidiophores of *U. cantlous* are distinctly longer than those of *U. botrytis* and *U. consortiale*. In addition this species is distinct in producing some fusiform conidia (both base and apex of conidia are subacute to conical) (FIG. 1A–D), whereas *U. botrytis* and *U. consortiale* do not. *U. consortiale* sometimes produces an apical secondary conidiophore approximately 25–35 × 4.0–4.5 µm with 5–8 conidiogenous sites, but secondary conidiophores of *U. cantlous* are only up to 5–10 µm long with 1–2 conidiogenous sites. *U. cantlous* does not produce multiplex conidia in different growth-stages under standardized conditions, whereas this character is typical for the *U. atrum* group.

To aid in the phylogenetic characterization of *Ulocladium* sp. isolated from *Cucumis* sp., the Alt a 1 (447 bases) and *gpd* (575 bases) regions of two isolates of *U. cantlous* were sequenced and compared with sequences from other *Ulocladium* sp. Results of the partition-homogeneity test ($P = 0.273$) indicated that the Alt a 1 and *gpd* genes trees reflect the same underlying phylogeny. Therefore these two datasets were combined and analyzed. In addition the Alt a 1 and *gpd* regions of *U. oudemansii*, *U. obovoideum* and *U. subcucurbitae*, as well as Alt a 1 regions of *U. capsicuma*, were sequenced. Phylogenetic relationships among the two *U. cantlous* isolates and other *Ulocladium* sp. were estimated with combined sequences of Alt a 1 and *gpd* (1034 sites).

A neighbor joining (NJ) tree was constructed based on combined sequences of *gpd* and Alt a 1 of *U. cantlous* and related species, with *Stemphylium botryosum* as outgroup (FIG. 2). The combined tree shows that a monophyletic *Ulocladium* species group comprises 13 *Ulocladium* species, *A. cheiranthi* and *E. indefessa*. This group includes two distinct sister clades. Clade 1, supported by a high bootstrap value (100%), includes the two isolates of *U. cantlous* together with *U. atrum*, *U. botrytis*, *U. cucurbitae*, *U. consortiale*, *U. subcucurbitae*, *U. multiforme*, *U. dauci* and *U. obovoideum*. Clade 2, which has low bootstrap support (52%), includes four *Ulocladium* species, *E. indefessa* and *A. cheiranthi*. One *Ulocladium* sp. (CBS 123375) is morphologically similar to *U. alternariae*, and these two species form a separate clade that has little commonality with other species of *Ulocladium*. The NJ analysis supports a distinction between *U. cantlous* and species *U. botrytis*, *U. consortiale* and others in this genus. In Clade 1 the *U. atrum* group (*U. atrum*, *U. cucurbitae*, *U. multiforme* and *U. dauci*) do not form an independent subclade; only *U. atrum* and *U. multiforme* cluster together with a low bootstrap value (64%). *U. cucurbitae* shows a high similarity to *U. obovoideum* supported by a moderate bootstrap value (88%). *U. subcucurbitae* and *U. dauci* occur on separate branches from the other species in Clade 1.

In the MP analysis two equally most parsimonious trees were obtained with tree length (TL) 384 steps, consistency index (CI) = 0.8464, retention index (RI)

TABLE II. Distinguishing characteristics of this new species and similar well known species of *Ulocladium* under ideal growth conditions

Species		Conidia					Dimensions of conidiophores (µm, length × width at base)
		Shape	Size (µm)	Transverse septa	Longitudinal or oblique septa	Conidial wall	
U. cantlous (CBS 123376)		obovoid or broadly ellipsoidal	24–36 × 13–15 (29 ± 3.7 × 14 ± 0.4)	3–4	0–2	verrucose to densely verrucose	80–130 × 3–4 (105 × 3.5 ± 0.2)
U. atrum (CBS 195.67)	1–3 d	broad-ellipsoid	29–33 × 16–20 (31 ± 1.5 × 18 ± 1.1)	3–4	1–4	smooth	80–100 × 5–7 (93 × 5.8 ± 0.6)
	4–7 d	obovoid or subglobose	18 × 22–16 × 18 (19.5 ± 1.2 × 17 ± 0.6)	1–3	1–2	strongly ornamented	
U. botrytis (CBS 198.67)		obovoid to broadly ellipsoidal	18–28 × 12–16 (23.5 ± 1.3 × 14 ± 0.6)	2–3	2–4	densely verrucose	60–95 × 4.0–4.5 (75 × 4.2 ± 0.2)
U. consortiale (CBS 123806)		obovoid or narrow ellipsoidal	22–40 × 11–15 (33 ± 4.1 × 12 ± 0.8)	3–4	3–4	closely micromaculate to definitely verrucose	60–100 × 3.5–4.5 (85 × 4 ± 0.3)
U. cucurbitae (CBS 483.81)	1–3 d	long-ellipsoid or cylindrical	34–41 × 11–12 (37 ± 1.1 × 11.6 ± 0.2)	6–7	1–2	smooth to punctate	45–70 × 4.5–5.0 (60 × 4.7 ± 0.2)
	4–7 d	obovoid or spherical	22–26 × 10–13 (24.5 ± 0.3 × 11.5 ± 0.4)	2–3	1–3	punctulate to verrucose to variously tuberculate	
U. dauci (CBS 102062)	1–3 d	broad-ovoid or ellipsoid	23–31 × 9–11 (26 ± 2.8 × 10 ± 0.6)	3–5	0–3	punctulate to tuberculate to pustulate	50–65 × 4.5–5.0 (57 × 4.6 ± 0.2)
	4–7 d	obovoid or spherical	20–24 × 13–15 (23 ± 0.9 × 14 ± 0.6) 16–20 (17.8 ± 1.5)	1–2	1–2	densely tuberculate to pustulate	
U. multiforme (CBS 102060)	1–3 d	narrow-ovoid/-ellipsoid	20–36 × 8–10 (30 ± 4.9 × 9 ± 0.8)	3–4	1–2	densely ornamented	50–80 × 4.5–5.5 (67 × 4.9 ± 0.3)
	4–7 d	obovoid or spherical	22–26 × 14–18 (24 ± 1.8 × 16.5 ± 1.3) 14–20 (16.5 ± 2.8)	1–2	1–2	densely tuberculate to pustulate	
U. obovoideum (CBS 101229)		broadly obovoid with a conspicuously pointed base	23–30 × 15–18 (27 ± 2.9 × 16.8 ± 0.9)	1–4	1–3	smooth	85–110 × 4.5–5.5 (96 × 5 ± 0.3)
U. subcucurbitae (CBS 121491)	1–3 d	obclavate or long-ellipsoid	28–48 × 11–14 (39 ± 4.5 × 12.5 ± 1.3)	3–6	0–2	smooth	28–58 × 4.0–5.0 (45 × 4.3 ± 0.3)
	4–7 d	long-ellipsoid or obclavate	28–63 × 11–16 (46 ± 11.5 × 13 ± 2.0)	4–7	0–4	smooth to pustulate	

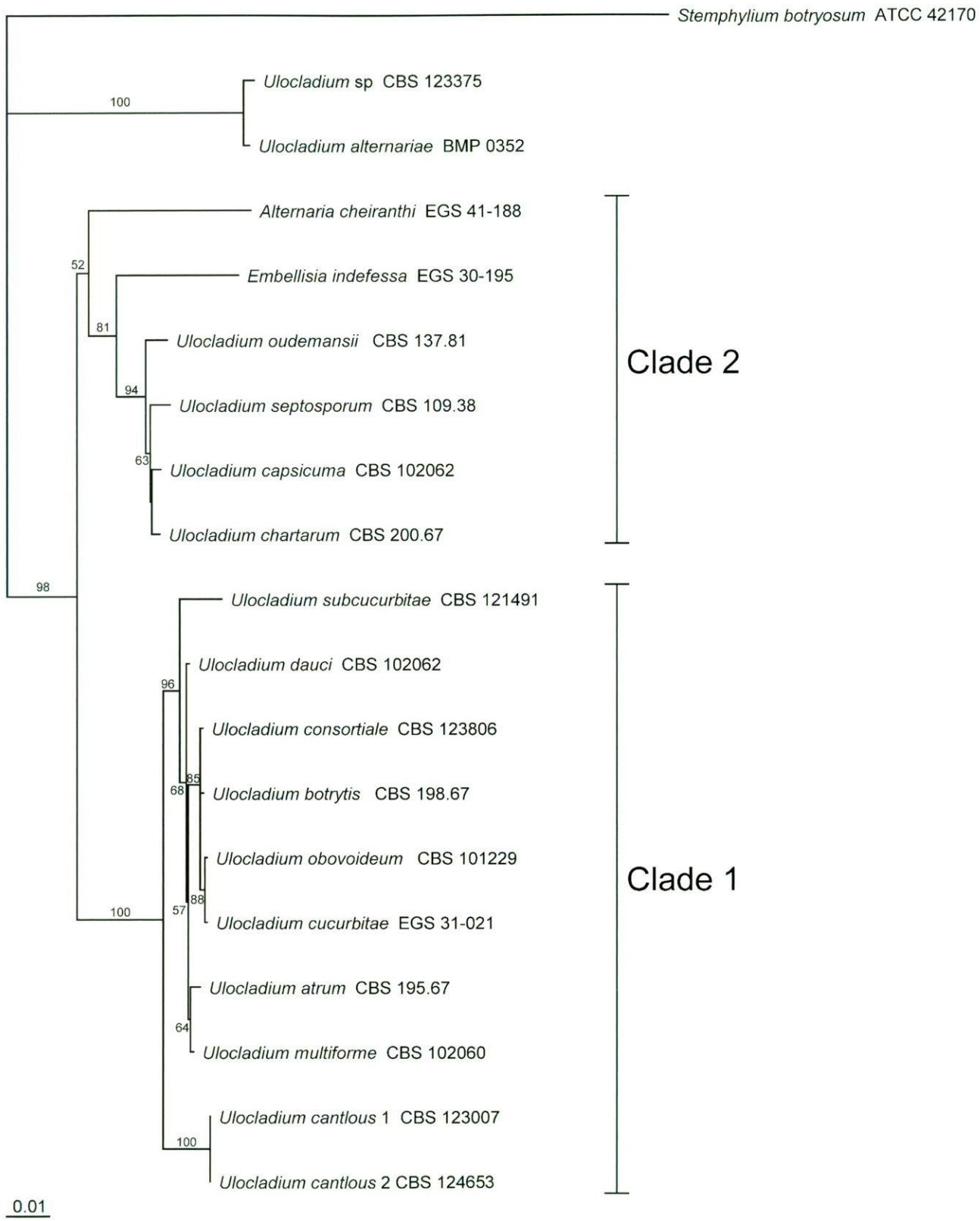

FIG. 2. Phylogenetic tree generated from neighbor joining analysis based on combined Alt a 1 and *gpd* sequences. Bootstrap support for the nodes is indicated above branches. Bootstrap values ≥ 50% are indicated above the branches. The tree is rooted with *Stemphylium botryosum*.

= 0.8589, rescaled consistency index (RC) = 0.7269 and homoplasy index (HI) = 0.1536. The phylogenetic tree from MP analyses indicates that all *Ulocladium* species in this study, except *U. alternariae* and *Ulocladium* sp. (CBS 123375), cluster together as a large group that is divided into two clades (FIG. 3). Two isolates of *U. cantlous* reside in a distinct subclade with no clear affinities to a specific sister group in Clade 1, which is composed of nine species of *Ulocladium* and has high bootstrap support (100%). In this clade the four species of the *U. atrum* group are not supported as an independent subclade. Clade 2, including *U. chartarum*, *U. capsicuma*, *U. oudemansii* and *U. septosporum*, is supported by higher bootstrap values (93%) than NJ analyses (52%). *E. indefessa* grouped with *U. chartarum* and *A. cheiranthi* in Hong et al. (2005), Pryor and Bigelow (2003) but is excluded from the *Ulocladium* group in the MP analysis. *A. cheiranthi* forms a sister clade outside Clade 1 and Clade 2 with moderate bootstrap support (83%). *Ulocladium* sp. and *U. alternariae* are placed outside the main *Ulocladium* species group, as in the NJ analysis, but each occurs on a separate branch in the MP analysis.

DISCUSSION

Ulocladium cantlous is similar to *U. atrum*, *U. botrytis*, *U. consortiale*, *U. cucurbitae*, *U. dauci*, *U. multiforme*, *U. obovoideum* and *U. subcucurbitae* with which it shares characteristics of conidiophores and conidia. It most resembles *U. botrytis* and *U. consortiale* in having obovoid or ellipsoid conidia but differs from these species mainly in the size, septation and ornamentation of conidia as well as the dimensions of conidiophores (TABLE II). Phylogenetic analysis reveals the evolutionary relationships among this and other species of *Ulocladium*. In the phylogenetic trees produced by NJ and MP (FIGS. 2, 3) two isolates of *U. cantlous* clustered in a separate subclade with a high bootstrap value (100%) that had no clear affinities to a specific sister group within the larger clade (Clade 1), which includes nine species of *Ulocladium*. Clade 1 probably represents the core of this genus characterized by key morphological characteristics (conidiophore development closely geniculate and conidia primarily obovoid). The phylogenetic results are generally consistent with above morphological comparison but do not support a closer relationship between this species and *U. botrytis* and *U. consortiale* than to other species in Clade 1. This suggests that the shape of conidia is not a reliable indicator of phylogenetic relationships in this genus. In addition multiplex conidium morphology is not present in this species under standardized conditions. The conidial characters at 1–3 d are similar to those at 4–7d (FIG. 1A–D), which distinguishes *U. cantlous* from four other species in the *U. atrum* group. Therefore both morphological and molecular evidence support the designation of a new species in this genus.

Multiplex conidium morphology is predicted to be of value in defining species related to the *U. atrum* group (Simmons 1998). This study attempts to validate the feasibility of this approach by molecular phylogenetic analyses. In phylogenetic trees based on NJ and MP (FIGS. 2, 3) *U. atrum* clusters with *U. multiforme* but *U. cucurbitae* is more closely related to *U. botrytis*. *U. dauci* formed a distinct subclade in Clade 1. The use of multiplex conidium morphology in identifying species in *U. atrum* group therefore might not reflect the evolutionary relationships among these four species.

Pryor and Bigelow (2003) recognized that the *Ulocladium* group included four *Ulocladium* species (*U. botrytis*, *U. atrum*, *U. consortiale* and *U. chartarum*), *E. indefessa* and *A. cheiranthi* by phylogenetic analysis of ITS/5.8S/ITS2, mtSSU and *gpd* gene regions. This conclusion was supported by Hong et al. (2005) who further demonstrated that this group was composed of two distinct sister clades, *U. atrum*, *U. botrytis*, and *U. cucurbitae* in one clade and *U. chartarum*, *E. indefessa*, *A. cheiranthi* in the other clade, based on the phylogenetic analysis of *gpd* and Alt a 1 gene regions. In this study additional species of *Ulocladium* were sequenced in the Alt a 1 and *gpd* regions to improve phylogenetic accuracy. Results from analysis of the two loci show that the *Ulocladium* group is divided into two distinct clusters (FIGS. 2, 3); Clade 1 includes nine *Ulocladium* species, while Clade 2 includes four species of *Ulocladium*. We suggest that these two clades are monophyletic, as supported by high bootstrap values in the NJ and MP analysis. Species in Clade 1 with high bootstrap (100%) in the phylogenetic analysis determined by NJ and MP possess the typical morphological characters of *Ulocladium*, which probably represent the core of this genus. The position of this clade as a large group is well defined by taxonomic and molecular phylogenetic studies. However the phylogenetic positions of *A. cheiranthi* and *E. indefessa* are not clearly resolved in this study because the NJ analysis is not congruent with MP analysis. The difference caused by two statistical approaches might be due to the small number of *Ulocladium* taxa investigated. In summary a comprehensive examination of morphological description under standardized condition and molecular analysis based on appropriate phylogenetic markers and methods are proved to be essential in identifying *Ulocladium* isolates.

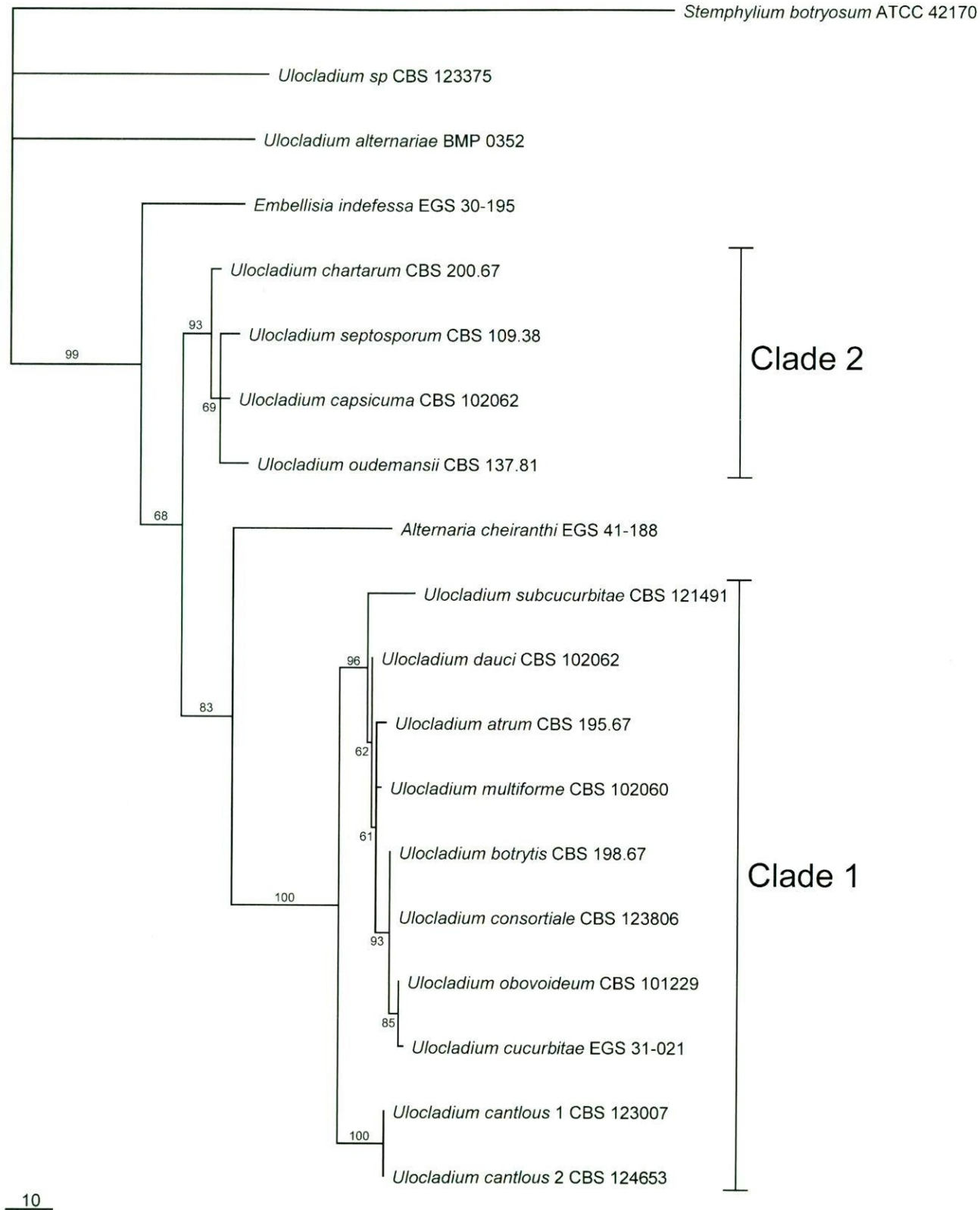

FIG. 3. Maximum parsimony tree derived from analysis of the combined Alt a 1 and *gpd* sequences. Bootstrap support for the nodes is indicated above branches. Bootstrap values ≥ 50% are indicated above the branches. The tree is rooted with *Stemphylium botryosum*.

ACKNOWLEDGMENTS

We thank the culture collection of the Centraalbureau voor Schimmelcultures for *U. cucurbitae*, *U. obovoideum* and *U. oudemansii* isolates. This work was supported by the National Natural Science Foundation of China (Grant 30570006).

LITERATURE CITED

Barnes CS, Pacheco F, Landuyt J, Rosenthal D, Hu F, Portnoy J. 1996. Production of a recombinant protein from *Alternaria* containing the reported N-terminal of the Alt a 1 allergen. In: Sehon A, Kraft D, Hay Glass KT, eds. Advances in experimental medicine and biology 409. New York: Plenum Press. p 197–203.

Berbee ML, Pirseyedi M, Hubbard S. 1999. *Cochliobolus* phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91:964–977.

Câmara MPS, O'Neill NR, van Berkum P. 2002. Phylogeny of *Stemphylium* spp. based on ITS and glyceraldehydes-3-phosphate dehydrogenase gene sequences. Mycologia 94:660–672.

Cramer RA, Lawrence CB. 2003. Cloning of a gene encoding an Alt a 1 isoallergen differentially expressed by the necrotrophic fungus *Alternaria brassicicola* during *Arabidopsis* infection. Appl Environ Microbiol 69:2361–2364.

———, ———. 2004. Identification of *Alternaria brassicicola* genes expressed in plant during pathogenesis of *Arabidopsis thaliana*. Fungal Genet Biol 41:115–128.

de Hoog GS, Horré R. 2002. Molecular taxonomy of the *Alternaria* and *Ulocladium* species from humans and their identification in the routine laboratory. Mycoses 45:259–276.

De Vouge MW, Thanker AM, Curran IHA, Zhang L, Muradia G, Rode H, Vijay HM. 1996. Isolation and expression of a cDNA encoding on *Alternaria alternata* Alt a 1 subunit. Int Arch Allergy Immun 111:385–395.

Elmer PAG, Köhl J. 1998. The survival and saprophytic competitive ability of the *Botrytis* spp. antagonist *Ulocladium atrum* in lily canopies. Eur J Plant Pathol 104:435–477.

Farris JS, Kallersjo M, Kluge AG, Bult C. 1995. Testing significance of incongruence. Cladistics 10:315–319.

Grishkan I, Beharav A, Kirzhner V, Nevo E. 2007. Adaptive spatiotemporal distribution of soil microfungi in Evolution Canyon 2, Nahal Shaharut, extreme southern Negev Desert, Israel. Bot J Linn Soc 90:263–277.

Hong SG, Cramer RA, Lawrence CB, Pryor BM. 2005. Alt a 1 allergen homologs from *Alternaria* and related taxa: analysis of phylogenetic content and secondary structure. Fungal Genet Biol 42:119–129.

Huelsenbeck JP, Bull JJ, Cunningham CW. 1996. Combining data in phylogenetic analysis. Trends Ecol Evol 11: 152–158.

Köhl J, Molhoek WWL, Goossen-van de Geijn HM, Lombaers van der Plas CH. 2003. Potential of *Ulocladium atrum* for biocontrol of onion leaf spot through suppression of sporulation of *Botrytis* spp. BioControl 48:349–359.

Leach CM, Aragaki M. 1970. Effect of temperature on conidium characteristics of *Ulocladium chartarum* and *Stemphylium floridanum*. Mycologia 62:1071–1076.

Preuss CGT. 1851. Die Pilze Deutschlands. Heft. 30. In Jacob Sturm's Deutschlands Flora, Abt III:73–96.

Pryor BM, Bigelow DM. 2003. Molecular characterization of *Embellisia* and *Nimbya* species and their relationship to *Alternaria*, *Ulocladium* and *Stemphylium*. Mycologia 95: 1139–1152.

———, Gilbertson RL. 2000. Molecular phylogenetic relationships among *Alternaria* species and related fungi based on analysis of nuclear ITS and mtSSU rDNA sequences. Mycol Res 104:1312–1321.

Runa F, Park MS, Pryor BM. 2009. *Ulocladium* systematics revisited: phylogeny and taxonomic status. Mycol Progress 8:35–47.

Sáenz-de-Santamaría M, Postigo I, Gutierrez-Rodríguez A, Cardona G, Guisantes JA, Asturias J, Martínez J. 2005. The major allergen of *Alternaria alternata* (Alt a 1) is expressed in other members of the Pleosporaceae family. Mycoses 49:91–95.

Saparrat MCN, Arambarri AM, Balatti PA. 2007. Growth response and extracellular enzyme activity of *Ulocladium botrytis* LPSC 813 cultured on carboxy-methycellulose under a pH range. Biol Fertility Soils 44:383–386.

Simmons EG. 1967. Typification of *Alternaria*, *Stemphylium*, and *Ulocladium*. Mycologia 59:67–92.

———. 1982. *Alternaria* themes and variations (11–13). Mycotaxon 14:44–57.

———. 1990. *Alternaria* themes and variations (27–53). Mycotaxon 37:79–119.

———. 1998. Multiplex conidium morphology in species of the *Ulocladium atrum* group. Can J Bot 76:1553–1559.

———, Roberts RG. 1993. *Alternaria* themes and variations (73). Mycotaxon 48:109–140.

Smith TL. 1989. Disparate evolution of yeasts and filamentous fungi indicated by phylogenetic analysis of glyceraldehydes-3-phosphate dehydrogenase genes. Proc Natl Acad Sci USA 86:7063–7066.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustsal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882.

Vannini A, Vettraino AM. 2000. *Ulocladium chartarum* as the causal agent of a leaf necrosis on *Quercus pubescens*. Forest Pathol 30:297–303.

Xue F, Zhang XG. 2007. *Ulocladium capsicuma*, a new species identified by morphological and molecular phylogenetic data. Sydowia 59:161–178.

Zhang G, Berbee ML. 2001. *Pyrenophora* phylogenetics inferred from ITS and glyceraldehydes-3-phosphate dehydrogenase gene sequences. Mycologia 93:1048–1063.

Zitter TA, Hsu LW. 1990. A leaf spot of cucumber caused by *Ulocladium cucurbitae* in New York. Plant Dis 74:824–827.